REVERSE MICELLE AS MEMBRANE MIMETIC AGENT - A STUDY OF CHOLESTEROL

SOLUBILIZATION IN WATER-AEROSOL OT-ISO-OCTANE SYSTEM

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Cholesterol is solubilized and condensed in the monomolecular hydrophobic shell of AOT reverse micellar droplet; the degree of condensation increases with increase in concentration of cholesterol. Saturation is attained when the concentration of cholesterol is 50% of the surfactant in the system. The condensation is a spontaneous process and the extent of saturation is surprisingly the same as observed in cholesterol-biological membrane system. The present study suggests that the surfactant monolayer of w/o microemulsion droplet can be used well as a model for biological membranes for studies of solubilization of extramembraneous components.

INTRODUCTION

Interaction of membranes with extramembrane components has been widely studied in model systems such as monolayers, bilayers and black lipid membranes, micelles, liposomes and other synthetic vesicles. Membrane mimetic studies have been successfully used to explain many biological phenomena like membrane-cholesterol interaction and condensation, absorption and transportation of fat soluble vitamins through biological membranes, and mechanism of anesthesia, but they have not reported on the nature of interaction at molecular levels.

Because of the anisotropic characteristics of the amphiphile aggregates, dispersed monolayers, bilayers, black lipid membranes and similar liquid crystalline systems cannot be successfully used to study the microscopic behavior as sometimes the experimental techniques demand isotropic nature of the solute particles. On the other hand, isotropic aggregated systems like micelles, vesicles, etc. have different and non-uniform packing of the amphiphiles so that the solubilization of extramembrane components into these aggregates follows neither the same solubilization kinetics as in the bilayer biological membranes nor uniform distribution of solubilizes into the hydrophobic regions of the amphiphilic assemblies. These constraints prompted us to
consider the use of surfactant monolayers of water-in-oil microemulsion droplets as solubilizing media for the purpose of studying the molecular level interaction of membranes because these droplets are more or less isotropic and the amphiphilic molecules have packing parameters comparable to those in biological membranes. As a logical extension of our consideration we have undertaken a detailed thermodynamic study of cholesterol solubilization into AOT reverse micelles. Since membrane-cholesterol interactions have been most widely studied using different model membrane systems, a comparison of our results in the light of these observations would establish the validity of the use of surfactant monolayers of AOT reverse micelles as an effective model membrane system for studying solubilization of extramembraneous components into membranes.

EXPERIMENTAL

(a) Materials and purification: Aerosol OT, i.e., sodium bis(ethylhexyl)sulphosuccinate (Fluka AG) was of pharmaceutical grade purity and was additionally purified by the method described elsewhere. Cholesterol of AR grade was recrystallized from ethanol and vacuum dried. Isooctane was distilled over calcium hydride before use.

(b) Solubilization diagrams: The cloud points and solubilization temperatures were measured on 0.1 molar AOT solutions in isooctane (and with added cholesterol as the case may be) containing various amounts of water according to the procedure described elsewhere. Solubilization diagrams were drawn by plotting upper and lower temperatures corresponding to turbid to clear and then clear to turbid, respectively, at various $W_o$ (= molar ratio of water to AOT).

(c) Interfacial tension measurements were made by the drop volume method and mean headgroup areas of AOT molecules ($A_{\text{HKO}}$) were calculated according to the procedure described in the literature.

(d) Partial specific volumes were determined from density measurements using digital densitimeter model PAAR DMA 601.

RESULTS AND DISCUSSION

Solubilization diagrams indicating clear microemulsion domains of water-AOT-isooctane systems with and without added cholesterol at various $R(=\text{Cholesterol}/[\text{AOT}])$ in which temperature of the system has been plotted against the molar ratio of water to AOT ($W_o$) are shown in Figure 1. For each system, a characteristic triangular shaped region is displayed which is conventionally considered to be the optically isotropic, clear, low viscous liquid, thermodynamically stable, the so-called water-in-oil (w/o) microemulsion region. The curves elucidate the influence of solubilization of cholesterol into AOT reverse micelles on their microemulsion stability. Figure 2 depicts that on gradual increase of cholesterol ($R$), the water solubilization capacity of AOT reverse micelles increases up to $R = 0.5$ and above which it remains practically constant.